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A single pulse of diffuse contaminants alters the size distribution of natural phytoplankton communities

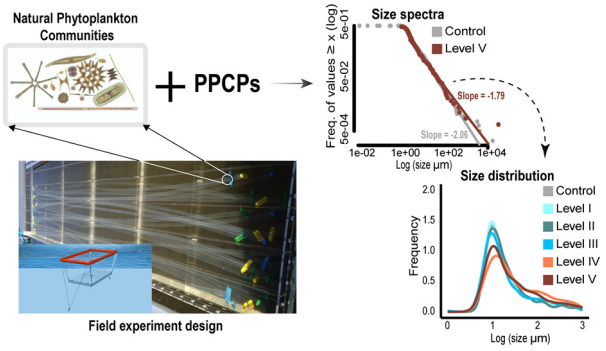
Didier L. Baho [a,*], Francesco Pomati [b,c], Eva Leu [d], Dag O. Hessen [e], S. Jannicke Moe [a], Jon Norberg [f], Luca Nizzetto [a,g]

[a] Norwegian Institute for Water Research (NIVA), CIENS, Science Park, Gaustadalleen 21, 0349 Oslo, Norway
[b] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Aquatic Ecology, Dübendorf, Switzerland
[c] Swiss Federal Institute of Technology (ETH) Zürich, Institute of Integrative Biology, Zürich, Switzerland
[d] Akvaplan-niva, CIENS, Science Park, Gaustadalleen 21, 0349 Oslo, Norway
[e] University of Oslo, Dept. Biosciences, Box 1066, Blindern, 0316 Oslo, Norway
[f] Stockholm Resilience Centre, Stockholm University, Kräftriket 2B, SE-10691, Stockholm, Sweden
[g] RECETOX, Masarik University, Kamenice 753/5, 625 00 Brno, Czech Republic

HIGHLIGHTS

- Ecological consequences of diffuse contaminants in natural systems are still scarce.
- PPCPs affect the scaling of abundances relative to organisms' size in phytoplankton.
- Vulnerability of smaller phytoplankton to contaminants resulted in structural changes.
- Changes in the phytoplankton community composition were similarly observed.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of a multitude of bioactive organic pollutants collectively classified as pharmaceuticals and personal care products (PPCPs) in freshwaters is of concern, considering that ecological assessments of their potential impacts on natural systems are still scarce. In this field experiment we tested whether a single pulse exposure to a mixture of 12 pharmaceuticals and personal care products, which are commonly found in European inland waters, can influence the size distributions of natural lake phytoplankton communities. Size is one of the most influential determinants of community structure and functioning, particularly in planktonic communities and food webs. Using an in-situ microcosm approach, phytoplankton communities in two lakes with different nutrient levels (mesotrophic and eutrophic) were exposed to a concentration gradient of the PPCPs mixture at five levels. We tested whether sub-lethal PPCPs doses affect the scaling of organisms' abundances with their size, and the slope of these size spectra, which describe changes in the abundances of small relative to large phytoplankton. Our results showed that a large proportion (approximately 80%) of the dataset followed a power-law distribution, thus suggesting evidence of scale invariance of abundances, as expected in steady state ecosystems. PPCPs were however found to induce significant changes in the size spectra and community structure of natural phytoplankton assemblages. The two highest treatment levels of PPCPs were associated with decreased abundance of

* Corresponding author.
E-mail address: didier.baho@niva.no (D.L. Baho).

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the most dominant size class (nano-phytoplankton: 2–5 μm), leading to a flattening of the size spectra slope. These results suggest that a pulse exposure to PPCPs induce changes that potentially lead to unsteady ecosystem states and cascading effects in the aquatic food webs, by favoring larger non-edible algae at the expense of small edible species. We propose higher susceptibility due to higher surface to volume ratio in small species as the likely cause of these structural changes.

1. Introduction

The widespread occurrence of anthropogenic chemicals in freshwater ecosystems is a global concern (Monteiro and Boxall, 2010; Rockström et al., 2009). Among these is a diverse range of pharmaceuticals and personal care products that reach surface waters predominantly by treated and untreated sewage (Daughton and Ternes, 1999). The extent of freshwaters contamination by PPCPs is predicted to increase due to multiple socioeconomic factors: the global increase of mega-cities (UN-Habitat, 2016) and a growing demand of pharmaceuticals (Vos et al., 2017), while the operational cost of advanced wastewater treatment technology to remove PPCPs can be a deterrent (Matamoros et al., 2007; Ternes et al., 2003). Pharmaceuticals are of particular interest as they are engineered to be biologically active and trigger desired therapeutic effects at low doses, which increases their likelihood to interact with non-target aquatic organisms (Grzesiuk et al., 2018). Despite these concerns, ecologically relevant investigations of PPCPs in natural settings are still scarce and ecosystem effects poorly understood (Bernhardt et al., 2017; Richmond et al., 2017). At the cellular level, exposure to PPCPs can hinder metabolic pathways related to lipids and chlorophyll-a synthesis (Zhang et al., 2012, 2019), which can transcend to higher organizational levels causing alterations in phytoplankton community structure (Lee et al., 2016; Pomati et al., 2017; Rosi et al., 2018). Inferring ecological effects of PPCPs under realistic low doses in natural environments is challenging due to the spatial and temporal variations of diverse aquatic taxa, hierarchical organization of ecosystems (individuals-species-populations-communities), and a broad range of variable environmental conditions (Rubach et al., 2011; Segner, 2011; Van den Brink et al., 2011; Van Straalen and Van Gestel, 2008). These challenges can be overcome by focusing on species traits, such as size (Rubach et al., 2011; Segner, 2011). Using trait-based approaches reduces data dimensionality (i.e. making systems comparisons easier) and can potentially provide mechanistic insights regarding species and communities' response to environmental alterations (Frimpong and Angermeier, 2010; McGill et al., 2006). Shifts in trait structure as a response to environmental changes can be coupled to broader ecological processes, including ecosystem functioning (Acevedo-Trejos et al., 2015; Frimpong and Angermeier, 2010; Yvon-Durocher and Allen, 2012).

Body size is arguably the single most important characteristic of an organism (Acevedo-Trejos et al., 2015; White et al., 2007) as it relates to other facets of life-history and ecological processes including metabolism, development, resource requirements and trophic interactions (Brown et al., 2004; Elton, 1927; Peters, 1983). The relation between organisms' size and abundance has generally been conceptualized as a probability distribution of individual sizes from a given community (Blanchard et al., 2008; Gaedke, 1992; Jennings and Mackinson, 2003; Sheldon et al., 1972; Sprules et al., 1983) which later was termed "size spectra" (Kerr and Dickie, 2001). The slope of the size spectra is typically negative, reflecting the commonly observed inverse relationship between abundance of organisms and their size, where the abundance of larger organism rapidly declined (coined "the pyramid of number" by Elton (1927)). This inverse relationship between organisms' size and abundance can conform to a power law function, resembling other biological phenomena such as species rank abundance, connectivity across metabolic pathways (Ravasz et al., 2002) and species-area relationship (García Martín and Goldenfeld, 2006). The slope thus describes how organisms' abundance decreases with size (Brown et al., 2004; Yvon-Durocher et al., 2011) and provides insights on how energy and materials are partitioned within or across trophic levels (White et al., 2007). Interestingly, the slopes of size spectra in steady state aquatic ecosystems appear to converge to values of about −2, both in marine (Cavender-Bares et al., 2001; Marañón, 2015) and freshwater environment (Gaedke et al., 2004; Sprules and Barth, 2015).

Empirical evidence suggests that shifts in size spectra are indicative of anthropogenic stressors on aquatic communities, and has been used to evaluate the effects of over-fishing (Bianchi et al., 2000; Rice and Gislason, 1996), climate change (Yvon-Durocher et al., 2011) and land use management (Martínez et al., 2016). For instance, over-fishing induced changes in the size structure in marine ecosystems, where the slopes of size spectra were found to steepen when subjected to the deliberate over-exploitation of targeted larger organisms (Bianchi et al., 2000; Rice and Gislason, 1996).

In phytoplankton, cell size is a crucial determinant of the outcome competition for limiting resources and trophic interactions (Litchman and Klausmeier, 2008; Marañón, 2015). For instance, large cells have low surface-to-volume ratios and are less efficient than small cells in nutrient uptake and are therefore at a disadvantage under low nutrient conditions (Tambi et al., 2009). In contrast, large cells or colonies are less susceptible of being grazed than small cells (Barton et al., 2013; Marañón, 2015), due to gape limitations in zooplankton. Cell size is also a key property for absorption and transformation of contaminants (Del Vento and Dachs, 2009). Size also determines the vulnerability of aquatic organisms to anthropogenic chemical pollution (Echeveste et al., 2012; Echeveste et al., 2011; Kline and Pinckney, 2016; Othman et al., 2012) where smaller sizes are generally more susceptible (Del Vento and Dachs, 2009). This susceptibility can be attributed to their high surface to volume ratios, where the very same traits that make them competitively superior in nutrient poor environments could render them more vulnerable to contaminants (Findlay, 1972; Tambi et al., 2009). Phytoplankton communities are the basis of aquatic food webs and are comprised of diverse assemblages (Hutchinson, 1961) of unicellular organisms that span over a wide range of sizes, making them ideal to use size-based indicator such as size spectra analysis to uncover structural changes induced by diffuse contaminants. Furthermore, they have short generation time and respond quickly to a broad range of stressors (McCormick and Cairns, 1994).

The present study addresses the ecological effects of PPCPs on natural phytoplankton community structure in environmentally relevant scenarios. We studied how PPCPs affect the scaling of abundances relative to organisms' size (i.e. the size spectra) of algal assemblages. Using an in-situ experimental approach, phytoplankton communities were exposed to five different levels of a PPCPs mixture. Individual phytoplankton size measurements were regularly taken over the course of 3-week field experiment, using scanning flow-cytometry. The experiment was conducted simultaneously in two lakes with different nutrient status to account for different community compositions and abundances. Here we tested whether (i) size spectra change along a sub-lethal concentration gradient of PPCPs, suggesting that these micropollutants can alter fundamental properties of natural species' assemblages; and (ii) PPCP exposure mostly affects smaller phytoplankton (as expected), which would be indicative of potential cascading effects on energy transfer in food webs (i.e. reduction of small – edible algae relative to large – less edible ones).

2. Materials and method

2.1. Site

The field experiment was conducted in two lakes located in the vicinity of Oslo, Norway. The lakes were located in the same region (Fig. S1) and were chosen based on their similarities in ambient climate and physico-chemical parameters with respect to pH and dissolved organic carbon concentration, while differing in their nutrient status. Lake Gjersjøen (59° 79′ 67″ N, 10° 77′ 36″ W, 40 m above sea level) is a mesotrophic lake (total phosphorus appx. 10 μg/L), has a total surface area of 2.6 km^2 and provides drinking water for the county's residents (Xiao et al., 2014). Nearby Lake Årungen (59° 69′ 68″ N, 10° 73′ 95″ W, 33 m above sea level) is a eutrophic lake (total phosphorus ca 30 μg/L), has a total surface area of 1.2 km^2 and receives agricultural run-off (Sharma et al., 2008). The experiment was performed in wind-sheltered locations over three weeks (between June 9th and June 29th, 2016) when the lakes were thermally stratified (Fig. S2a-b). In-situ physico-chemical parameters (temperature, light and nutrient level) were regularly monitored, whereas prevailing climatic conditions (air temperature and precipitations) were obtained from the closest meteorological monitoring stations (see Table S1a-c and Fig. S3a-b). Permission to conduct the field experiment was formally obtained from their respective municipalities; Oppegård and Ås.

2.2. Experimental setup

Natural lake phytoplankton communities were exposed to a concentration gradient of a PPCPs mixture consisting of 12 compounds in semi-permeable dialysis bag and were incubated in-situ (Fig. 1, description of details below). The experimental design consisted of six treatment levels (one control and five PPCPs exposure levels each in triplicates), which were regularly sampled (six time points) during the experiment.

A "mixed" phytoplankton community was established by sampling 5 L from each successive meter from a water column of 10 m using a Limnos sampler. Samples were filtered through 60 μm nylon mesh, to exclude large zooplankton grazers, and subsequently pooled in a 50 L plastic carboy to obtain depth integrated community. All operations were conducted while preventing the phytoplankton from being exposed to solar radiations.

2.2.1. PPCPs exposure levels

The mixture of PPCPs used was based on a previous study performed by Pomati et al. (2017) reflecting environmentally relevant concentrations (Table 1) for most substances (with the exception of: Atenolol, Clarithromycin and benzophenone-4, for the highest exposure treatment; level V). These 12 substances are frequently detected in freshwaters (Table 1, Table S2a). The relative proportions of the 12 compounds were kept constant (to reflected monitoring data: Table 1) while performing dilutions to derive the respective treatment levels. In total, four serial dilutions were performed starting from the highest exposure level (V) to obtain a total of five treatment levels (I–V), which differed by a dilution factor of 2.7 (roughly the Euler's number) between each successive level. The solutions were prepared using dimethyl sulfoxide (DMSO) as a carrier solvent. In the highest treatment level (V), apart from clarithromycin, the concentrations of the other compounds were at least ten times lower than reported half-maximal effective concentrations (EC50) obtained from standard toxicity tests (Table S2b). Further details about the preparation of the mixture can be found in the Table S3.

2.2.2. Setting up microcosms using dialysis bags

Well-homogenized subsamples (908 mL) were taken from the carboy containing depth-integrated phytoplankton communities before exposing the communities to a concentration gradient of the PPCPs mixture. Treatments were spiked with 91 μL of the PPCPs mixture using five stock solutions of increasing concentrations (I–V), while the control was spiked with an equivalent volume of the carrier solvent (DMSO).

Immediately after spiking, a sub-sample of 158 mL was taken to determine conditions at day 0. The remaining 750 mL were carefully transferred into 2.5 m long cellulose ester dialysis bags with a flat-width of 31 mm (Spectra/por, Spectrum Europe, Breda, The Netherlands) and sealed with universal nylon clips (Spectra/por) at both ends (Fig. 1). The certified pore size of the dialysis bags ranges from 100 to 500 Da and allows the free exchange of nutrients, gases and compounds of similar or smaller molecular weight with the surroundings. The behavior of the 12 chemical compounds inside the dialysis bags was previously demonstrated by Pomati et al. (2017) to follow a pulse-like pattern, where the substances rapidly diffused out with half-lives ranging from few hours to few days. After 3–7 days the concentrations of contaminants inside the dialysis bags become negligible. Changes in phytoplankton community structures observed at the end of the experiment

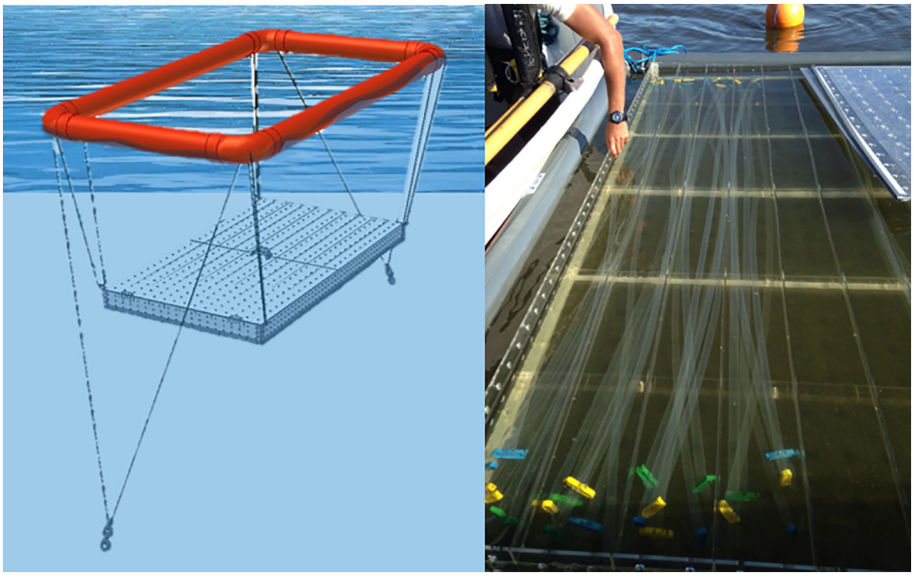

Fig. 1. The experimental setup (conceptual figure; left panel and real picture; right panel) consisting of a floating platform made of airtight PVC pipes which was anchored to the lake floor. A submersible acrylic rack was attached to the floating platform, which was used to protect the dialysis bags containing the phytoplankton communities and were lowered to the depth of maximum chlorophyll-a. The rack was raised to the surface during sampling.

Table 1
Nominal concentration of the PPCPs mixture (ng/L) modified from Pomati et al. (2017).

Chemical	Therapeutic category	Highest spiked concentration for level V	Median concentration found in European Rivers (Pomati et al., 2017)	NORMAN Monitoring database for European freshwaters[a]			
				Min	Max	Times analyzed	Percentage detection (%)
Atenolol	Anti-hypertensive	2700	351	0.1	900	977	74.0
Bezafibrate	Lipid regulator	270	46	0.3	21,200	1384	55.2
Carbamazepine	Anticonvulsant	2700	121	0.8	7600	22,270	86.9
Clarithromycin	Antibacterial	2700	143	0.9	1100	945	77.2
Diclofenac	Anti-inflammatory	2700	190	0.2	110,000	6320	70.2
Furosemide	Diuretic	270	49.5	0.5	283,000	507	16.6
Hydrochlorothiazide	Diuretic	2700	174	4.0	389,000	484	48.6
Ibuprofen	Anti-inflammatory	270	97	1.2	303,000	5154	71.2
Ranitidine	Ulcer healing	27	16.5	1.3	200	50	58.0
Sulfamethoxazole	Antibacterial	27	7	0.7	700	2616	81.5
Benzophenone-4	Solar filter	2700	178	NR	NR	NR	NR
Triclosan	Antibacterial	270	59	1	3060	11,565	78.3

[a] Data downloaded from the online database of the NORMAN network: http://www.norman-network.net.

can therefore be attributed to trans-generational effects of the parental compounds and/or secondary metabolites. The optical and hydrophilic properties of the dialysis bags ensure transparency for photosynthetic active radiation (PAR) and reduce biofouling. As shown in previous studies, these bags allow isolating and studying phytoplankton in a realistic environmental setting (Pomati et al., 2017; Pomati and Nizzetto, 2013). Once sealed, the dialysis bags were placed in a custom-designed submersible protective acrylic (transparent to PAR) rectangular cuboid rack (length: 2710 mm, width: 1540 mm, height: 150 mm) and incubated at 2.5 and 3 m below the surface, in Gjersjøen and Årungen, respectively. The incubation depths corresponded to the depth of maximum chlorophyll-a concentration (Gjersjøen: 3 μg/L and Årungen: 5 μg/L). The rack consisted of different chambers to segregate dialysis bags of different treatments and was attached to a floating platform (made of PVC pipes) that was anchored at a fixed position (see Fig. 1).

2.3. Sampling procedure

Samples were taken from each replicate every fourth day during the course of the experiment (20 days) to determine individual cell sizes from the phytoplankton communities. The plexiglass frame (Fig. 1) was brought to the surface and covered by dark plastic sheets to minimize light induced stress during sampling. Samples were obtained by cutting a predetermined length of the dialysis based on the length/volume conversion (3.1 mL/cm) provided by the manufacturer, after gently massaging the dialysis bags for homogenization. 10 mL from each replicate were collected to determine size. The samples (10 mL) were fixed with a solution of paraformaldehyde and glutaraldehyde (respective concentrations of 0.01 and 0.1%, pH 7 and total volume of 100 μL) and stored in the dark at 4 °C prior to analysis using scanning flow cytometry.

Samples for species determination were collected and analyzed for two time points: the beginning (day 0) and at the end of the experiment (day 20). 50 mL were collected, from each replicate that was fixed with lugol's solution (0.5 mL).

2.4. Scanning flow-cytometry

A scanning flow-cytometer from Cytobuoy (Woerden, the Netherlands; http://www.cytobuoy.com) was used to count and characterize size distribution of phytoplankton at an individual level. The device is equipped with two solid-state lasers (488 nm and 635 nm) and designed to analyze phytoplankton ranging from picoplankton to larger phytoplankton (0.5 to 700 μm in diameter and about 1 mm in length). Size of individual phytoplankton cells was determined by scattering, where the light scattered from particles (≥1 μm in length) while passing the lasers beams was measured at two different angles; forward (FWS) and sideward scatter (SWS). The fluorescence (FL) emitted by photosynthetic pigments from algal cells were measured at four different wavelengths: red (FLR), green (FLG), orange (FLO) and yellow (FLY). The raw Cytobuoy data were processed with R statistical computing software using distribution of FL as a filter to retain FL particles relevant for phytoplankton with a size ≥1 μm in length. More details about on Cytobuoy analysis can be found elsewhere (Fontana et al., 2018; Thomas et al., 2018).

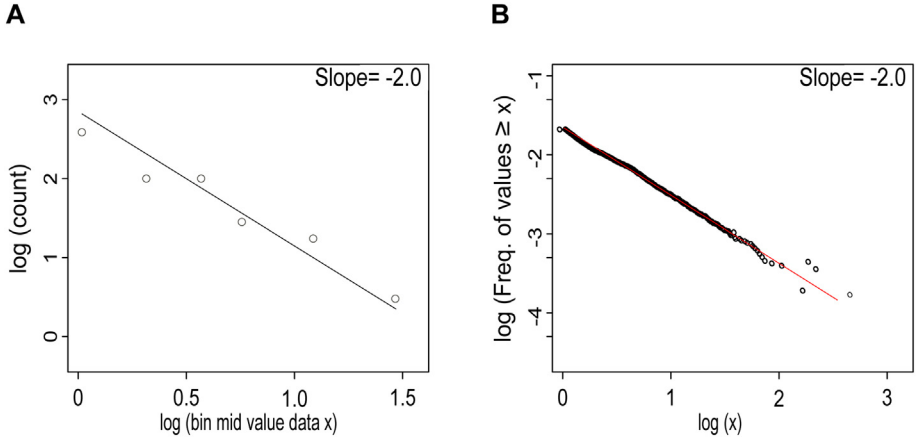

Fig. 2. Conceptual figures indicating different methods, normalized abundance spectra (A) and Type I Pareto probability density function (B), generally used to fit size-abundance distribution to estimate size spectra (Modified from Edwards et al. (2017)).

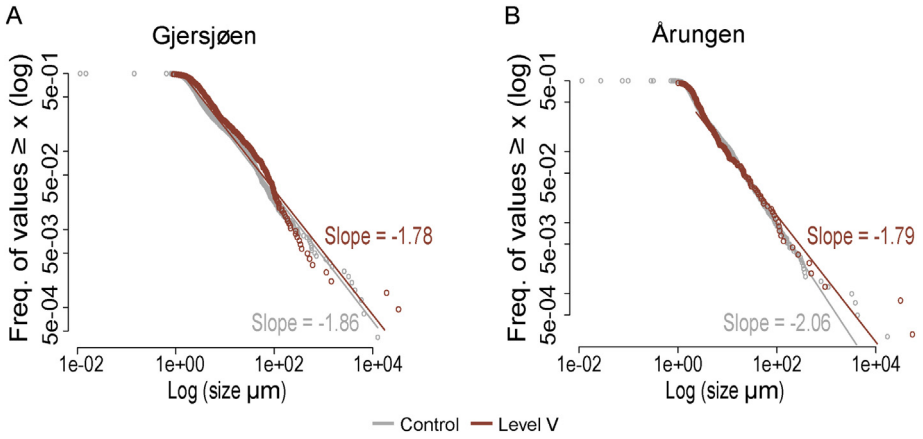

Fig. 3. Community size spectra comparing the control to the highest treatment (level V) for Gjersjøen (A–B) and Årungen (C–D) for the last sampling point: day 20.

2.5. Species determination

Phytoplankton was identified by a trained taxonomist using the Utermöhl technique, generally to the species level, and biomass (mg/m^3) was calculated from geometric conversion based on a standard protocol (CEN - EN 16695). Only the control was analyzed for the starting point (day 0), as we did not expect an immediate change in species composition after a few hours preceding the addition of diffuse contaminants.

2.6. Statistical analyses

Statistical analyses were performed using R statistical (version 3.3.1) software (R Core Team, 2017).

2.6.1. Size classes and size spectra

The scale specific effects of PPCPs on the size distribution of phytoplankton across lakes was tracked by assigning the entire dataset (for each treatment and each time point) to seven classes (2–5, 5–10, 10–20, 20–50, 50–100, 100–200, >200 µm). These size classes were chosen to provide a better resolution compared to the two broad traditional size classes: nano 2–20 µm and micro 20–200 µm phytoplankton (Sieburth et al., 1978).

Conventionally, community size spectra are depicted as a function of the normalized size to abundance, which are confined to regular size class intervals (through data binning), resulting in a straight line where the slope (Fig. 2a) can be estimated (Quintana et al., 2002). However, binning dependent methods can introduce researchers' subjectivity due to the lack of guidance to select the number of bins, which is known to influence how size spectra are derived (Edwards et al., 2017; Martínez et al., 2016; Sprules and Barth, 2015). This methodological pitfall can be avoided by defining size spectra as a Type I Pareto probability density function, where community size spectrum $s(l)$ is defined as the probability that an individual sampled randomly from the community has size l. The defined probability density $s(l)$ can be fitted to a power law function $s(l) \propto l^{-n}$, where the exponent -n is conceptually equivalent to the slope of size spectra (Fig. 2b).

The distribution can be fitted using maximum likelihood methods to derive a parameter that is equivalent to the size-spectrum slope, which is conventionally depicted by other methods (Edwards et al., 2017). The maximum likelihood approach, unlike the other methods, does not require binning (Edwards et al., 2017) and gives better fits (Vidondo et al., 1997). Size spectra were constructed for each treatment and time sampling point using the poweRlaw package developed for R (Gillespie, 2015). The goodness of fit to the maximum likelihood methods was evaluated using Kolmogorov-Smirnoff test, p-values (<0.1); replicates that did not follow a power-law distribution were excluded from statistical analysis. Similarly, the initial data points (day 0) were excluded from the analyses to avoid possible influence of mixing communities along the water column. Size spectra data from the replicates (that followed a power-law distribution) were analyzed using a multiple regression analysis to determine which of the fixed factors (treatment, time, lake (as categorical variable) and treatment – time interaction), had significant effects and predictions were based on the model's coefficients. The model selection was based on Akaike's Information Criteria to compare other alternatives. A Type III, unweighted mean analysis, was implemented in the final model to account for the unequal number of observations as some replicates were excluded from the analysis. Assumptions of normality and homoscedasticity were visually assessed using the residuals of the model.

2.6.2. Species composition

The effects of PPCPs on the community composition were investigated using a multivariate ordination technique: principal coordinate analyses, which preserved the direct relationships among treatments (Borcard et al., 2018). Principal coordinate analyses were performed using square root transformed species and Bray-Curtis dissimilarity matrix. The principal coordinate analyses were complemented with permutational multivariate ANOVA (PERMANOVA), using the same dissimilarity matrix (Bray-Curtis) and transformed species data with 9999 permutation and an α-level of 0.05.

Table 2
Summary of the effects of treatment, time, lakes and treatment: time interaction term on phytoplankton size spectra. Bold values correspond to significant P-values at 0.05.

Effects	Sum of squares	F value	*P*
Treatment	**0.41**	**8.21**	**<0.01**
Time	0.02	0.32	0.57
Lake	**21.76**	**218.81**	**<0.01**
Treatment: Time	**0.37**	**7.54**	**<0.01**

Table 3
Coefficients of the multiple regression analysis performed on the size spectra data, significant predictors (P-values at 0.05) are highlighted in bold. The overall model was significant ($p < 0.01$) with an adjusted R^2 value of 0.988.

Predictor	*B*	*SE. B*	t value	*P*
Treatment	**−0.08**	**0.03**	**−2.87**	**<0.01**
Time	0.02	0.03	0.56	0.57
Treatment: Time	**0.02**	**0.008**	**−2.75**	**<0.01**

3. Results and discussion

3.1. Effect on size spectra

A large proportion of the dataset followed a power law distribution, 74% and 87% for Gjersjøen and Årungen, respectively. The proportions that did not follow a power law distribution showed no consistent pattern that could be related to treatment or temporal effects (Fig. S4). The slopes of community size spectra displaying a power law distribution of sizes, ranged from −1.58 to −2.79 in Gjersjøen (mesotrophic lake) and from-1.58 to −2.96 in Årungen (eutrophic lake). The size spectra changed towards shallower slopes (i.e. decrease in smaller relative to larger cells; Fig. 3 and Fig. S5), when exposed to the PPCPs mixture. Treatment, lake type and the interaction term (treatment x time) were found to have significant effects on size spectra (Table 2). In contrast, time did not have a significant effect on size spectra. The coefficients of the multiple regression model (Table 3) showed that increasing the concentration of PPCPs significantly decreased the steepness of the slope, whereas time and the interaction term (treatment x time) had the opposite effect (with only the interaction term being significant). Prior studies have demonstrated that size spectra of freshwater aquatic communities can shift when subjected to anthropogenic stressors (Martínez et al., 2016; Yvon-Durocher et al., 2011).

3.2. Effect on size distributions

The size distribution (Fig. 4) and size classes analyses (Fig. 5, Fig. S6a-b) revealed that phytoplankton belonging to the nano size class (2–5 μm) was predominant in both lakes, collectively representing approximately 65% of all algae abundances (data not shown). The high prevalence of nano-plankton (2–5 μm) drove the overall response of the community (Fig. 5). The total abundance of phytoplankton in all treatments (including controls) was found to consistently decrease until day 8. This initial drop in abundance might not be dependent on the chemical stress imposed by PPCPs and could be related to a bag effect. However, after 12 days the abundance of phytoplankton in the controls recovered whereas the abundances in the two highest treatment levels kept decreasing (Fig. 5). Previous studies (Lamichhane et al., 2014; Länge et al., 2010) reported similar time-lag effects of pharmaceuticals at sub-lethal concentrations, which only became apparent after multiple generations. The ability of PPCPs to induce transgenerational effects highlights the importance of addressing time-dependency along ecologically relevant scales. Exposure to the two highest treatment levels (IV, V; Fig. 4c, d and Fig. S7) triggered a shifts in size distributions, where the average size was observed to increase compared to the control (level IV: Gjersjøen: 180.9 μm: Årungen: 256.3 μm, level V: Gjersjøen: 256.3 μm: Årungen: 198.9 μm – control: Gjersjøen: 29.5 μm, Årungen: 14.0 μm). In contrast, the effects of the lower treatments (I-III) were negligible. In both lakes, the dominant (small) size class was consistently found to be more susceptible to increasing concentrations of PPCPs compared to the other size classes (Fig. 5 and Fig. S6a-b).

3.3. Community composition

The two lakes had different phytoplankton community composition (Fig. 6), Bacillariophyceae (diatoms) was the dominant group in lake Gjersjøen whereas Cryptophyceae (cryptomonads) were initially dominant in lake Årungen that later shifted to Cyanophyceae (blue-green algae). The taxonomic composition also changed noticeably during the experiment (Fig. 6). At the beginning of the experiment (day 0), the high prevalence of Cryptophyceae were generally representative of spring conditions in boreal lakes (Arvola, 1986), which changed by the end of the experiment (day 20). The presence of PPCPs was found to

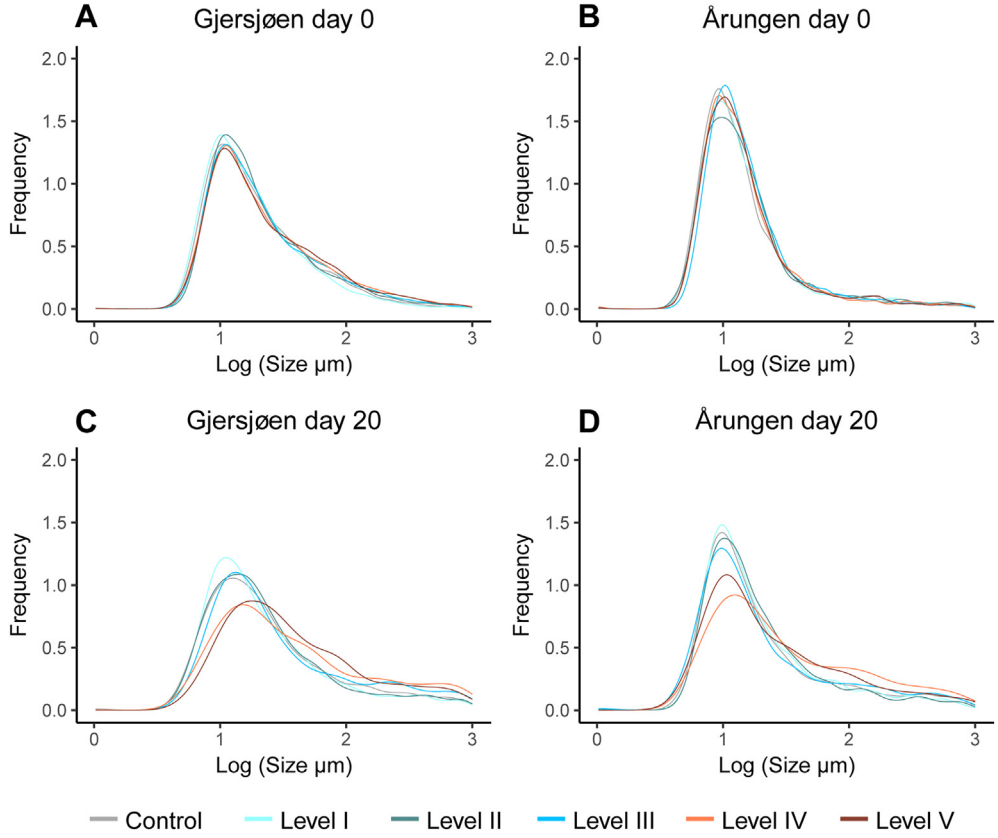

Fig. 4. Size distribution of the phytoplankton communities from the mesotrophic lake Gjersjøen (A–C) and the eutrophic lake Årungen (B–D) sampled, respectively, at the start and the end of the experiment across the control and treatments. The horizontal axis; size (μm) was scaled using common logarithm.

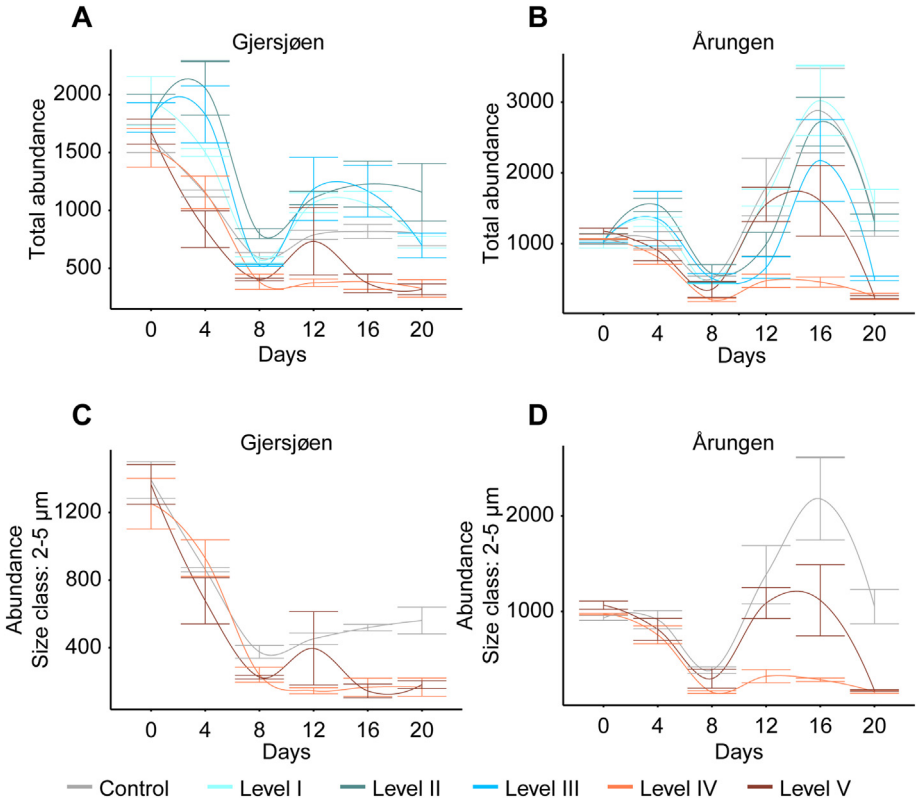

Fig. 5. Temporal changes in total abundances of phytoplankton compared to those belonging to size class (2–5 μm) from lake Gjersjøen (A, C) and lake Årungen (B, D) across treatments (colour coded). Treatment levels I–III, though not shown for the selected size class (2–5 μm) were close to the control. The vertical axes very not scaled and reflect differences between the lakes. Locally estimated scatterplot smoothing (LOESS) was fitted to the data points to generate line plots. The error bars indicate standard error.

induce changes in the community structure where the proportion of most taxa decreased with increasing exposure levels (Fig. 6). However, Cyanophyceae and Chrysophyceae were found to increase in relative abundance in the highest treatment level (level V) in Gjersjøen, whereas a similar pattern was observed in Årungen for unidentified taxa ("others") and Chrysophyceae. The ordination plots (Fig. 7) showed that in both lakes, the phytoplankton community compositions of the lower treatment levels (I and II) were undistinguishable from the controls. A gradual increase in dissimilarity was evident as from treatment level III and onward whereas more pronounced separation occurred in treatment level V. The results of the permutational multivariate ANOVA test provided addition support that PPCPs induced significant changes in the phytoplankton communities' structure (PERMANOVA: Gjersjøen $F_{5, 17} = 1.41$, $p < 0.05$, Årungen $F_{5, 17} = 1.95$,

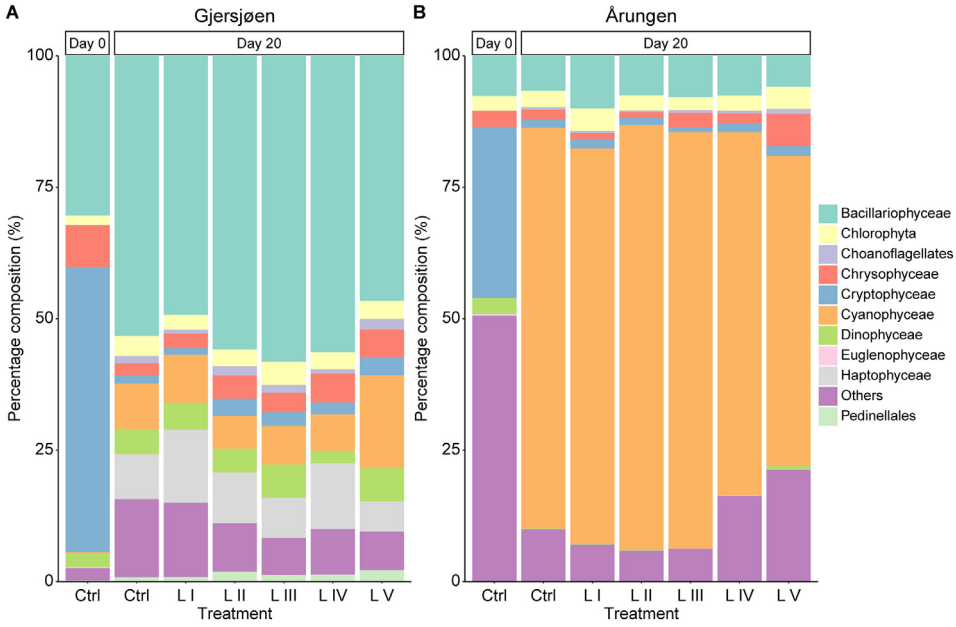

Fig. 6. Relative proportion of major phytoplankton classes identified in (A) Gjersjøen (mesotrophic lake) and (B) Årungen (eutrophic lake) showing: (i) changes in the controls that occurred between the start (day 0) and the end of the experiment (day 20) and (ii) the effects of on PPCPs treatments relative to the control at the end of the experiment (day 20).

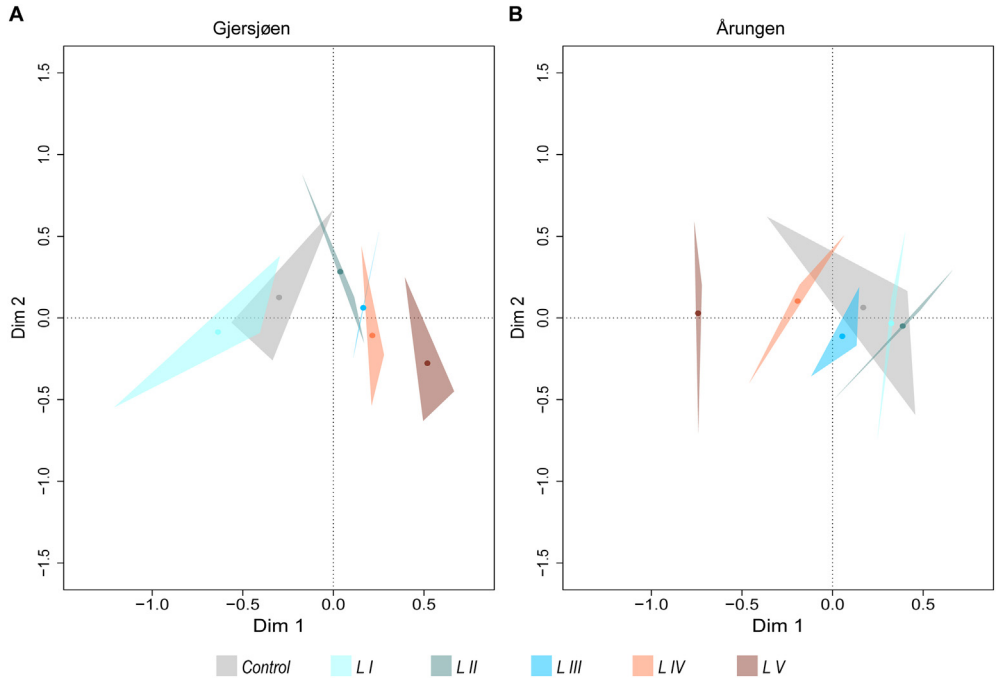

Fig. 7. Principal coordinate analysis biplots of the phytoplankton communities' species composition comparing the control and treatment levels for (A) Gjersjøen (mesotrophic lake) and (B) Årungen (eutrophic lake) by the end of the experiment (day 20). The corners of the polygons represent the replicates with their respective central positions (centroids) depicted by circles.

$p < 0.01$). Similar shifts in community composition triggered by PPCPs were found by others (Lee et al., 2016; Richmond et al., 2016; Wilson et al., 2003), favoring certain groups of phytoplankton species, such as cyanobacteria (Harris and Smith, 2016).

3.4. Relevance of size

Body size is a comprehensive descriptor of communities and captures key functional aspects that might be affected by anthropogenic stress (Acevedo-Trejos et al., 2015; Brown et al., 2004; Elton, 1927; Peters, 1983; White et al., 2007). The results presented here show for the first time that a pulse exposure to PPCPs alters the size spectra of natural phytoplankton communities and thereby provide new insights about the potential consequences of micropollutants on aquatic ecosystems.

Size mirrors the spatial-temporal scale at which species operates (Holling, 1992), with small organisms having faster growth rates, and shorter generation time than larger ones (Peterson et al., 1998; Villarino et al., 2018). Similarly, the effects of disturbances are claimed to be scale-specific and subsequently affect organisms that happen to operate within similar scales (Holling, 1992; Peterson et al., 1998). Our results suggest that PPCPs impact mostly the smaller size classes, which generally show faster growth rate and turnover time, as well as higher surface to volume ratios (Findlay, 1972; Litchman et al., 2009; Tambi et al., 2009). In our study, we observed a strong decline in the smallest fraction of cells (2–5 μm) at the highest treatment levels (IV and V; Fig. 5, Fig. S6a-b). Larger surface to volume ratio has been linked to faster dynamic of contaminant exchange and higher accumulation of pollutants through membranes (Del Vento and Dachs, 2009). The presence of micro-grazers inside the bags (verified during the taxonomic analysis) could have had influenced the abundance of smaller phytoplankton as they are known be selective feeders. However, the abundances of micro-grazers (Fig. S8) in the control were comparable to those from the treatments, which potentially indicates that the observed change in size spectra of algae could not be entirely driven by these organisms.

The impact of PPCPs exposure on the smaller size fraction caused the slope of the community size spectra to become shallower with increasing concentration of PPCPs. The effect of time (Table 2) was not significant, suggesting that the latter was less important than the treatment effect. In contrast, the interaction term (treatment x time; Table 2) had an opposite effect on size spectra. The interaction effect can be potentially linked to changing environmental conditions over time (Fig. S9). The nutrient drawdown (especially phosphorus: Fig. S9) is anticipated during summer thermal stratification, thereby increasing competition for limiting resources (Reynolds, 1976; Reynolds, 1980). Under low nutrient availability smaller phytoplankton are expected to have a competitive advantage over larger ones (Litchman et al., 2009; Tambi et al., 2009) However, since they are at the same time more vulnerable to PPCPs, which suggests a trade-off between competition and susceptibility to the contaminants.

3.5. Ecological implication

Changes in size-abundance relationships (Norkko et al., 2013; Yvon-Durocher and Allen, 2012) have been demonstrated to alter ecosystem functions (Allen et al., 2005; Barneche et al., 2014). Considering the important role of phytoplankton in carbon sequestration and nutrient cycling (Laws et al., 2000), shifts in community size spectra as a consequence of PPCPs exposure, have potentially far-reaching implications (Martínez et al., 2016) such responses were induced by a single pulse exposure event at sub-lethal levels. The negative effects of PPCPs on the most abundant size class of the phytoplankton community can potentially decrease the efficiency of the carbon transfer mediated the phytoplankton food web and shift the pathways towards heterotrophic bacteria via the microbial loop (Hlaili et al., 2014). Furthermore, (Grzesiuk et al., 2018) found that the effects of pharmaceuticals can propagate across trophic levels. Our results suggest that PPCPs can potentially alter the aquatic food web by shifting the edible portion that is appealing to grazer (Lampert, 1974) towards larger non-edible species, and can lead to blooms of larger phytoplankton (Moustaka-Gouni et al., 2006).

4. Conclusion

In summary, size spectra analysis allows comparisons of phytoplankton community responses from lakes with distinct trophic states and species composition. Through this analysis the effects of sublethal exposure to PPCPs were observed to change the fundamental relationship between size and abundance in two ecosystems. Our results complement the findings of other recent studies showing that PPCPs induce changes in phenotypic diversity (Pomati et al., 2017; Pomati and Nizzetto, 2013), community composition (Lee et al., 2016; Richmond et al., 2016; Wilson et al., 2003) and community metabolism (Wilson et al., 2004) of phytoplankton assemblages, thus supporting the claim that PPCPs should be considered as an environmental stressor of primary concerns (Richmond et al., 2017) - in particular considering their nearly ubiquitous occurrence in European freshwaters. Unlike other pollutants such as herbicides and pesticides that are applied at specific times (Rosi-Marshall and Royer, 2012), PPCPs are continuously released into aquatic recipients (Daughton and Ternes, 1999; Richmond et al., 2017). Our findings stress the urgent need to improve long-term exposure ecological assessments and to evaluate interactions with other co-occurring anthropogenic stressors in aquatic ecosystems (Côté et al., 2016; Galic et al., 2018; Rapport and Whitford, 1999). Such information can help to improve environmental management and policies to protect aquatic ecosystem and safeguard ecosystem services in a long-term perspective. This study showed that unifying and adapting concepts from macro-ecology to ecotoxicology can provide new insights and stimulate future research on the effects of diffuse chemical pollution at an ecosystem level.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.05.229.

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